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The Endocrine Pathway Responsible for Oocyte Maturation in the Inarticulate Brachiopod *Glottidia*

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Abstract. An aqueous extract of lophophore from *Glottidia pyramidata* induces oocyte maturation and follicle cell retraction in pieces of ovary and spawning in intact animals. The extract does not act directly on large oocytes but indirectly, through some other cell type in the ovary. The activity of the extract is insensitive to boiling or protease treatment and passes through a filter with a molecular weight cutoff of 2000. Bromoadenosine 3′5′ cyclic monophosphate and dibutyryl 3′5′ cyclic monophosphate have the same oocyte-maturing and spawning-inducing properties as lophophore extract. The active component in lophophore extract presumably affects somatic cells in the ovary, stimulating a rise in cyclic monophosphate levels in these cells, which then secrete a factor that causes oocyte maturation.

Introduction

Living inarticulate brachiopods consist of three families: the Lingulacea, the Discinacea, and the Craniacea. The Lingulacea is the only group for which our knowledge of reproductive biology can be considered more than rudimentary. During the breeding season, in the two genera (*Lingula* and *Glottidia*) that make up this family, each female spawns more than once, with intervals of several days between spawnings. This has been directly observed in individuals followed daily for several months in the laboratory (Chuang, 1959) and inferred from studies on the ovaries of females in a cohort collected at different times during the breeding season (Paine, 1963). The periodic presence of young larvae in plankton tows, a few days after the large tidal fluctuations around full moon, suggests that spawning normally occurs at this time in animals in their natural habitat (Yatsu, 1902; Paine, 1963). Chuang (1959) has challenged this interpretation; however, his plankton tows were taken so far apart in time that his data do not meaningfully address this issue. Animals also appear to spawn in response to environmental stress during transfer to the laboratory (Chuang, 1959; Kumé, 1956; Paine, 1963).

All previous work with embryos and young larvae of inarticulate brachiopods has been done on naturally spawned eggs. It is possible to obtain fertilizable oocytes from articulate brachiopods outside their normal spawning season by removing the ovary and macerating it to free the oocytes (Reed, 1987). As the oocytes stand in seawater, the follicle cell layer around each oocyte is retracted, the oocyte germinal vesicle breaks down spontaneously, and the chromosomes condense on the meiotic apparatus in preparation for the first meiotic reduction division. When the ovary of the inarticulate brachiopod *Glottidia pyramidata* is treated in the same way, the follicle cell layer is shed; however, the germinal vesicle does not break down, and the oocyte will not develop following the addition of sperm. This paper presents a method for inducing oocyte maturation in *G. pyramidata* and outlines a possible endocrine pathway that leads to oocyte maturation and spawning.

Materials and Methods

Animals

Specimens of *G. pyramidata* were collected during minus tides at Alligator Harbor, Florida, by digging them out of their burrows. Animals and their sediment were transported to the laboratory where they were maintained in a tray with sediment about 4 cm deep, with seawater flowing over it. Most animals oriented themselves in the sediment with their anterior end perpendicular to the sed-

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iment-seawater boundary and formed a three-hole slit typical of the entrance to their burrows. Animals fed and remained healthy under these conditions for at least a month. The valve lengths of the animals collected varied between 11 and 24 mm; all of these animals were sexually mature. Sexual maturity was assayed by noting the relative size of the gonad.

Tissue extracts

The effects of various tissue extracts on oocyte maturation were tested in a number of experiments. Aqueous extracts were made from a standard amount of adult *Glottidia* tissue. Because of their small size, more than one animal had to be used to obtain tissues for some experiments. After dissection, the tissue was blotted on a lint-free wipe, weighed, and broken up into small pieces with a watchmaker's forceps. One hundred microliters of distilled water was added to each 10 mg of tissue, and the tissue was homogenized with a tight-fitting pestle for 5 min in a 1.6-ml microcentrifuge tube. After homogenization, the tube was centrifuged or allowed to stand until the tissue debris had settled on the bottom; the supernatant was decanted and saved. In many experiments, all or part of the supernatant was boiled for 15 min prior to use. Tissue extracts were tested for biological activity by adding one part of a tissue extract to three parts of pasteurized seawater (PSW) and incubating pieces of gonad (*ca.* 1 mm^3) in this solution or in 1/2 serial dilutions of this solution in PSW. PSW was made by filtering seawater through a 0.45-μm Millipore filter and heating the seawater for 15 min above 80°C, but below boiling.

Culture procedures, ovary dissociation, and oocyte marking

All experiments were carried out at room temperature (21–24°C). Pieces of gonad were dissociated into single large oocytes and clusters of somatic cells by washing them three times and incubating them in calcium-free seawater for 90–120 min. (Calcium-free seawater consists of 425 m*M* NaCl, 9.4 m*M* KCl, 22.1 m*M* $MgCl_2$, 25.6 m*M* $MgSO_4$, 2.1 m*M* $NaHCO_3$ and 10 m*M* TES at pH 7.8–8.0.) At the end of this period, the gonad fragments were transferred to PSW and dissociated by pipetting the pieces up and down through a small-bore pipette. A 1% solution of the dye neutral red was prepared in distilled water. Five microliters of dye was added to 1 ml of PSW. Full-grown oocytes were incubated in this stain dilution for 5 min and washed in PSW. Stained oocytes can be distinguished from unstained oocytes for several hours because of their red coloration.

Preparation of motile sperm and fertilization

In some experiments, sperm were added to oocytes to see if the treatment would induce maturation and to eggs that had undergone maturation to see if they could be fertilized. A small piece of testis was dissected out, macerated in 1 ml of PSW, and diluted in about 100 ml of PSW. Most *Glottidia* sperm prepared in this way are nonmotile or move very slowly. Rapid sperm motility was induced by adding one drop of 1 *M* NH_4OH to the diluted sperm. One drop of the diluted sperm was added to oocytes or eggs in 1 ml of PSW.

Histology

Pieces of ovary were fixed in 1% osmium in PSW at 1–3°C for one hour, stored in 70% ethanol, dehydrated through an alcohol series, transferred to propylene oxide, and embedded in Epon equivalent. Sections were cut at 2 μ*M* and stained with methylene blue and azure II. Pieces of ovary, isolated immature oocytes, and mature oocytes were fixed in 5% paraformaldehyde in PSW at 1–3°C for 10–30 min, transferred to PSW, and stained with the blue fluorescing DNA stain DAPI to visualize nuclei. A stock solution of DAPI was prepared by dissolving 1 mg of stain in 10 ml of distilled water and diluted 1/25 in PSW prior to the addition of fixed pieces of ovarian tissue or oocytes.

Results

Ovarian structure

The structure of the *Lingula* ovary has been described by Senn (1934) at the light microscope level and by Sawada (1973) at the electron microscope level of resolution. During the breeding season, the ovaries of a sexually mature *G. pyramidata* consist mostly, on a volume basis, of full-grown oocytes; however, intermingled with the full-grown oocytes are a number of previtellogenic oocytes and oocytes that are engaged in vitellogenesis but are not yet fully grown. Figure 1A shows a whole mount of a piece of fixed ovary viewed with transmitted light. The full-grown oocytes, each with its large germinal vesicle, are obvious. In Figure 1B the same piece of ovary is shown, using fluorescence to visualize DNA. The DNA stain is evident in the nuclei of follicle cells that surround each oocyte. The chromatin in germinal vesicles of large oocytes does not stain because it is too diffuse. Figure 2 presents a cross-section through a piece of ovary at low and high magnification, showing various details of ovarian structure. Each full-grown oocyte is surrounded by an extracellular envelope with oocyte microvilli extending into the envelope. The follicle cells cover this envelope. In addition to follicle cells, the interstices between oocytes may contain at least one other kind of somatic cell that may be similar to a cell type that Sawada refers to as a nutritive cell and that Senn refers to as a nurse cell. According to Sawada and Senn, this cell type is prominent during early stages of oogenesis prior to the reproductive season but

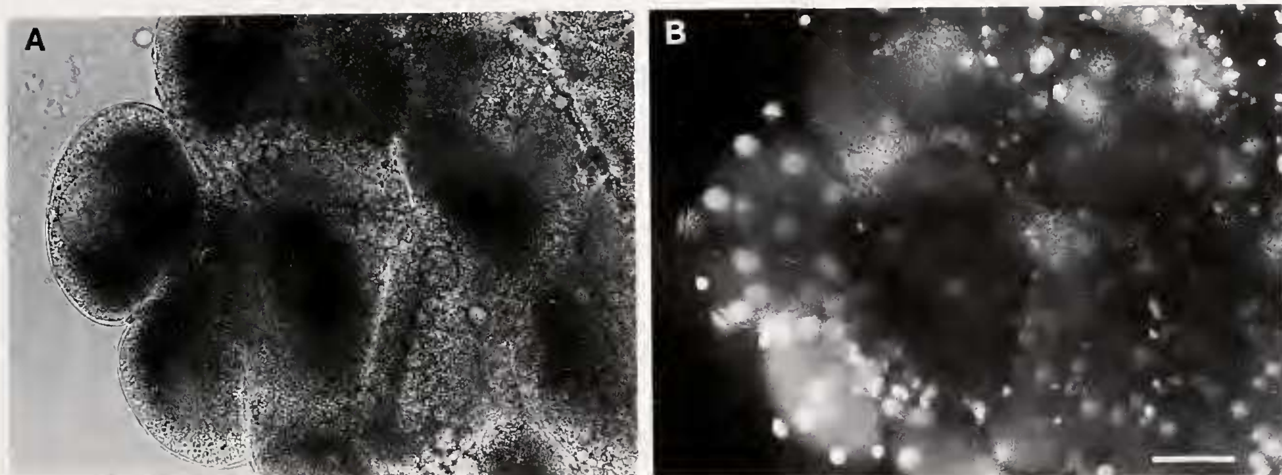

Figure 1. (A) Whole mount of ovarian lobe viewed with transmitted light showing full-grown oocytes. The translucent region in the oocyte on the extreme left indicates the position of its germinal vesicle. (B) View of the same ovarian lobe using fluorescence to visualize the nuclei of somatic cells in the ovary. Each oocyte is surrounded by a number of follicle cells. Because of the thickness of this preparation, not all somatic cell nuclei are in focus. For this reason, many of them give a diffuse fluorescence image. Both photographs are at the same magnification. Scale bar = 50 μm.

disappears in mature gonads; I have not seen this cell type. The full-grown oocytes form a polarized epithelium within the ovary. The germinal vesicle is located in the region of each oocyte that faces the coelomic space of the gonad, and the envelope that surrounds the oocytes is always thinnest on the opposite side of the oocyte facing the interior of the gonad (see *a* in Fig. 2B).

If one attempts to obtain oocytes by mechanically macerating the ovary, virtually all the oocytes are physically damaged: the envelope that surrounds the oocyte tears at the site where it is thinnest, and cytoplasm leaks out. Intact, undamaged oocytes can be obtained by washing and incubating pieces of ovary in several changes of calcium-free seawater (see Materials and Methods). Under these conditions, the follicular epithelium around the oocyte retracts, and the oocytes can be dissociated from the ovary. Oocytes obtained in this manner do not have follicle cells on their surface; this point was checked by staining fixed oocytes with DAPI. These follicle-cell-free oocytes still have an extracellular envelope with microvilli extending into the envelope. If these oocytes are cultured in PSW for up to 8 h, the germinal vesicle remains intact. When

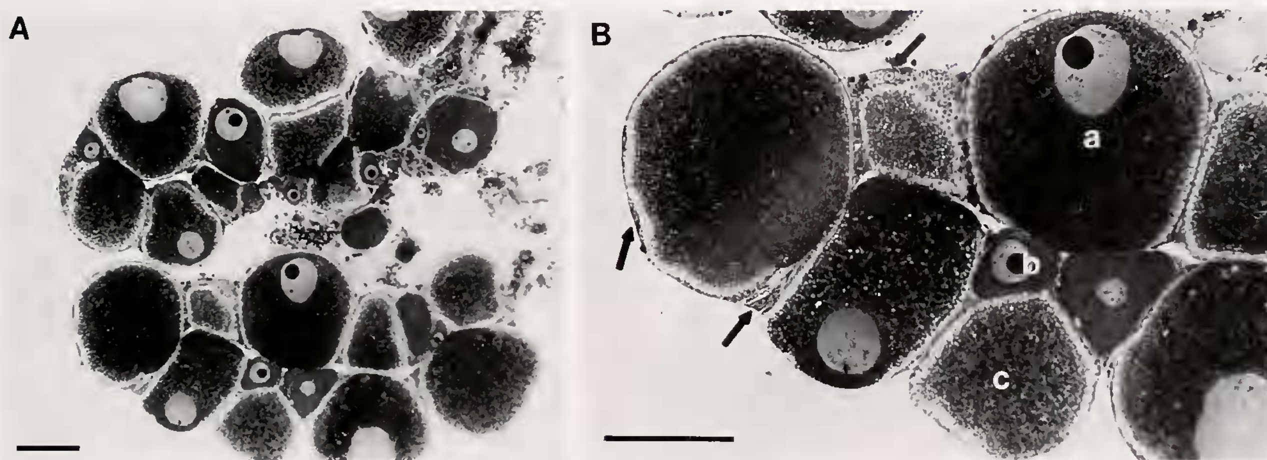

Figure 2. (A) Cross-section through two ovarian lobes at low magnification. The germinal vesicle of each oocyte has one nucleolus. (B) Cross-section through a region of a lobe at higher magnification. The envelope around each large oocyte and the follicle cells (arrows) covering the envelopes can be seen: (a) full grown oocyte, (b) previtellogenic oocyte, and (c) vitellogenic oocyte. Scale bars = 50 μm.

sperm are added to these oocytes, the germinal vesicle does not break down and development is not initiated. However, studies of fixed oocytes, stained with DAPI following exposure to sperm, indicate that sperm-egg fusion does occur in some cases.

The lophophore contains a factor that induces oocyte maturation

In a number of invertebrate and vertebrate animals, oocyte maturation in the ovary is controlled by hormones secreted by other organs (*e.g.*, Meijer, 1979a, b, for polychaetes and Shirai and Walker, 1988, for asteroids). Organ extracts from sexually mature *Glottidia* were tested for their capacity to induce oocyte maturation. Organ extracts were prepared from gut, liver, lophophore (including the mouth and the first part of esophagus), mantle, ovary, and testis (see Hyman, 1959, for a description of these organs). A piece of ovary was incubated in extract for up to 3 h and examined during this period for germinal vesicle breakdown of its oocytes. At the end of the incubation period, the piece of ovary was treated with calcium-free seawater to obtain free oocytes, and individual full-grown oocytes were examined for germinal vesicle breakdown by compressing them slightly under a coverslip and examining them with a compound microscope. At least three experiments were done for each organ, testing extracts of that organ from three animals. In the case of the gut, liver, lophophore, and mantle, extracts from both sexes were tested.

The only organ exhibiting oocyte-maturing activity was the lophophore. After 70–90 min of incubation in lophophore extract, the follicle cells around the oocyte retracted and the ovary dissociated into a pile of mature, full-grown oocytes and a residue composed of growing oocytes and follicle cells (Fig. 3). The germinal vesicles of these shed oocytes had broken down. DAPI staining of fixed oocytes showed that the chromosomes were condensed at metaphase and were located just under the cell surface in preparation for the first meiotic reduction division; there were no follicle cells around the oocytes. These matured oocytes were somewhat flattened, with a convex and concave side. When sperm are added to oocytes matured in this manner, the meiotic reduction divisions are completed and normal embryogenesis occurs.

In most experiments, an extract of 10 mg of lophophore in 100 μl of distilled water is still capable of inducing oocyte maturation when diluted 1/128 with PSW, but not when it is diluted to 1/256. Figure 4 summarizes the results of a number of experiments in which the last one-half dilution in a series that induced maturation is recorded. There was no sex-based difference in the concentration of oocyte-maturing activity in the lophophore.

The lophophore is a complex structure (Hyman, 1959; Storch and Welsch, 1976). There is a concentration of nerve cells, the subenteric ganglion, around the esophagus just below the mouth; nerve cell processes extend into the tentacular arms of the lophophore. To find out if a special region of the lophophore produces oocyte-maturing factor, the lophophore of a large animal was divided into two regions: the area around the mouth, which included the subenteric ganglion, and the two arms of the lophophore, which lack the subenteric ganglion. Extracts from both regions had the same oocyte-maturing activity (two experiments).

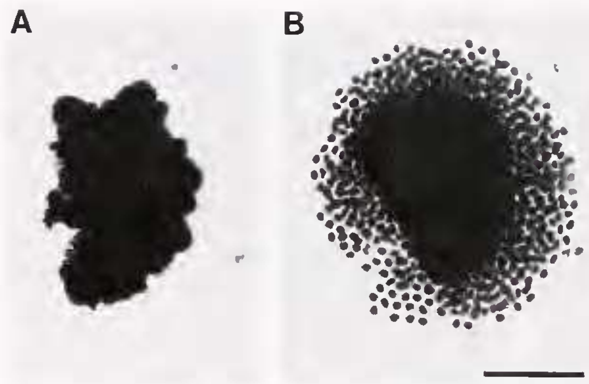

Figure 3. (A) A piece of ovary viewed with transmitted light just after being placed in a well with a 1/128 dilution of lophophore extract. (B) The same piece 95 min later showing its dissociation into a pile of large oocytes. Both photographs are at the same magnification. Scale bar = 1 mm.

Characterization of the lophophore extract

Three procedures were used to characterize the lophophore extract. In the first procedure, the extract was boiled to see if this treatment altered its activity. The results of a typical experiment are presented here. Lophophore extract was obtained from a male. Half of the supernatant was boiled for 15 min and the other half was stored on ice. A small amount of flocculent precipitate, presumably denatured protein, forms on boiling. Immediately after boiling, the boiled and unboiled extracts were serially diluted by 1/2 and the titer of the lowest dilution that would promote oocyte maturation was determined to be 1/128 for both boiled and unboiled extract. The remainder of the undiluted boiled and unboiled extract was frozen and retested for oocyte-maturing activity 24 h later. The lowest dilution of either extract that would induce maturation was 1/8, indicating that although lophophore extract is not heat labile, it loses activity in solution. Similar results were obtained using lophophore extract from either male or female animals in three separate experiments.

The second procedure examined the effect of protease treatment on the activity of lophophore extract. Extract

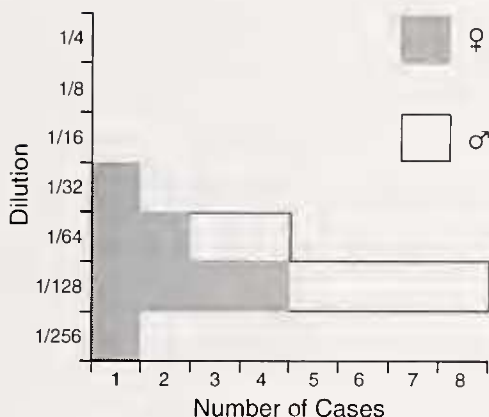

Figure 4. Histogram indicating the lowest 1/2 dilution of lophophore extract from a population of animals that induces oocyte maturation and follicle cell retraction in a piece of ovary. Each unit is one individual.

was boiled and diluted 1/32 with PSW. Then protease bound to agarose beads (Type VIII-A, 2–3 units per ml, Sigma) was slowly added to part of the diluted extract under a dissecting microscope until the solution could not accept any more. After 60 min of incubation at room temperature, the solution was centrifuged and the treated supernatant was collected. The last 1/2 dilution that would induce oocyte maturation in pieces of gonad was then compared for protease-treated and untreated extracts. In one experiment, this dilution was 1/128 for both extracts. In another experiment, it was 1/128 for the untreated extract and 1/64 for the protease-treated extract.

The third procedure characterized the lophophore extract by determining whether it would pass through a filter (Amicon) with a molecular weight cutoff of *ca.* 2000. Extract was boiled and diluted 1/32 with PSW prior to filtration. The lowest dilution of unfiltered solution that induced maturation in gonad pieces was 1/128, whereas the lowest dilution of filtered solution that induced maturation was 1/64. Because a large number of lophophores had to be dissected out and homogenized, this experiment was done only once.

These experiments indicate that the oocyte-maturing activity of the lophophore is due to a relatively small molecule and that it is probably not a peptide. The last part of the conclusion should be viewed with caution, however, because some small peptides can show marked resistance to boiling and protease activity.

The role of cAMP in inducing oocyte maturation

In many animals the levels of second messengers such as adenosine 3′5′ cyclic monophosphate (cAMP) changed markedly in oocytes or their follicle cells during oocyte maturation. In hydrozoans, an increase in cAMP levels in oocytes induces their maturation (Freeman and Ridgway, 1989). This compound was tested here to see if it would induce oocyte maturation in brachiopods. When animal cells are incubated in cAMP, they normally do not respond because this compound does not diffuse across the cell membrane, and there are no cAMP receptors on cell surfaces. For this reason, 8-bromoadenosine 3′5′ cyclic monophosphate (bromo cAMP) and dibutyryladenosine 3′5′ cyclic monophosphate (dibutyryl cAMP) were also tested; these cAMP derivatives can diffuse across cell membranes. In these experiments, summarized in Table I, a small piece of ovary was added to these compounds in PSW. The response of pieces of ovary to membrane-permeable cAMP derivatives was identical to their response to lophophore extract. Oocytes matured in this manner could be fertilized, and they developed into normal embryos. High concentrations of cAMP elicited only a marginal oocyte maturation response, but concentrations of bromo and dibutyryl cAMP that were 20–50 times lower were maximally effective in inducing maturation. The brominated derivative of 5′ adenosine monophosphate (bromo AMP), the breakdown product of cAMP, had no effect on oocyte maturation. These experiments show that cAMP derivatives induce oocyte maturation; however, they do not demonstrate that the oocyte-maturing activity in lophophore extract is a cAMP derivative.

Cyclic AMP derivatives and lophophore extract do not act directly on large oocytes to induce maturation

In a number of vertebrates and invertebrates, the neuroendocrine pathway that mediates oocyte maturation is made up of a number of components. For example, in asteroids, a factor from the radial nerve acts on the follicle cells that surround oocytes, causing them to secrete 1-methyl adenine, which then acts on the oocyte (Shirai and Walker, 1988). To find out if cAMP derivatives and

Table I

*The effect of different concentrations of cyclic AMP and cyclic AMP derivatives on oocyte maturation in pieces of gonad**

Compound	Conc m*M*	Maturation		
cAMP	100	±	±	−
	10	−	−	−
Bromo cAMP	5	+	+	+
	2	+	±	±
	1	±	−	−
Dibutyryl cAMP	5	+	+	+
	2	+	+	±
	1	±	±	±
Bromo AMP	20	−	−	−

* Each compound at each concentration was tested three times in experiments using pieces of ovary from different females. + = maturation, ± = marginal maturation, − = no maturation.

lophophore extract that cause maturation in pieces of isolated gonad will also cause isolated oocytes devoid of follicle cells to mature, pieces of gonad were incubated in calcium-free seawater (see Materials and Methods) to obtain isolated oocytes. These isolated oocytes were then treated with 5–10 mM of bromo or dibutyryl cAMP, or with 1/8 or 1/4 dilutions of lophophore extract in PSW; at the same time, pieces of ovary from the same animal were treated with the same concentrations of these agents. In every case oocyte maturation occurred in the intact piece of gonad, but in no case did it occur in oocytes devoid of follicle cells (117 from 7 animals). At the end of the experiment, the oocytes looked healthy and their germinal vesicles were intact.

The lack of maturation by isolated oocytes in the presence of cAMP derivatives or lophophore extract could reflect a subtle form of damage that the oocytes incurred during isolation. A reconstitution experiment was done to rule out this possibility. Isolated oocytes were stained lightly with neutral red so that they could be identified. These marked oocytes were placed either on or close to a piece of intact ovary that was then treated with 5–10 mM bromo cAMP, or with 1/4 or 1/64 dilutions of lophophore extract. This experiment was done three times. Of 46 stained oocytes, 28 underwent maturation as indicated by germinal vesicle breakdown; most of the 28 were in contact with the ovary. Maturation was first evident about 30 min after the ovary began to dissociate into oocytes. In control experiments, in which stained oocytes were placed on or close to a piece of ovary in the absence of bromo cAMP or lophophore extract, none of the 30 marked oocytes tested matured. These experiments suggest that cAMP derivatives or lophophore extract act in, or on, a somatic cell in the ovary, which then produces a factor that acts on large oocytes to mediate their maturation.

The oocyte-maturing activity in lophophore extract is part of an endocrine mechanism that mediates spawning

The oocyte-maturing activity in the lophophore could be a pharmacological fluke that has nothing to do with normal oocyte maturation. It could also be produced by a hormone that is released into the body cavity, where it functions as part of the normal endocrine pathway that mediates oocyte maturation and spawning.

During the night after animals are collected, they sometimes spawn (Paine, 1963). Spawning is obvious in females isolated individually in dishes without sediment, because one can see the eggs. The titer of oocyte-maturing activity in the lophophore of two females that spawned under these circumstances was tested the next morning by determining the lowest dilution by 1/2 in a series that induced oocyte maturation in pieces of ovary. In these experiments, the pieces of ovary used in the assay did not come from the females that had just spawned. The lowest dilutions that induced maturation were 1/8 and 1/16 for these two females. These results should be compared with the data on the lowest dilutions that normally induce maturation (Fig. 4). Two of the data points in Figure 4 come from females that were collected at the same time as the two females that spawned during the night. These two females did not spawn during the night. Lophophore extract from these females was prepared and tested at the same time the tests were done on the extracts from the two females that had spawned. The same set of ovarian fragments were used for all four tests. The lowest lophophore dilutions that induced maturation in these two cases were 1/64 and 1/128. This result indicates that just after a spawning there is much less oocyte-maturing activity in the lophophore. It suggests that the lophophore plays a role in normal spawning.

The breakdown of the germinal vesicle in oocytes and the dissociation of oocytes from a piece of isolated ovary do not constitute spawning. When spawning occurs in *Glottidia*, gametes are shed into the coelom, where they are expelled from the animal through its nephridiopores and pumped out of the shell. Spawning can be induced in these animals by injecting them with 25 μl of boiled lophophore extract diluted 1:3 with PSW or 25 μl of 20 mM dibutyryl cAMP in PSW. Large females with a valve length of 20 mm or more were used for these experiments. A 100-μl Hamilton syringe with a small-diameter hypodermic needle was used for the injections, which were made into the fluid-filled space that runs down the center of the pedicle. The pedicle, which is already partially contracted, contracts even more as a consequence of the injection, forcing the fluid in the center of the pedicle into the part of the animal covered by the valves. After the injection, each animal was placed in a separate dish with PSW and checked at intervals for eggs. Three of five animals injected with lophophore extract and two out of four animals injected with dibutyryl cAMP spawned. None of the five control animals injected with 25 μl of PSW spawned. Spawning began 2 to 3 h after the injection and lasted from 30 to 60 min. In each positive case, between 1000 and 5000 eggs were spawned. This experiment shows that lophophore extract and cAMP derivatives can elicit the entire maturation and spawning response in an intact animal.

Discussion

The fact that *Glottidia* spawns several times during its reproductive season and the observation that spawning is associated with the lunar cycle suggest that these animals may have an intrinsic mechanism for controlling the timing of spawning. The experiments reported here suggest

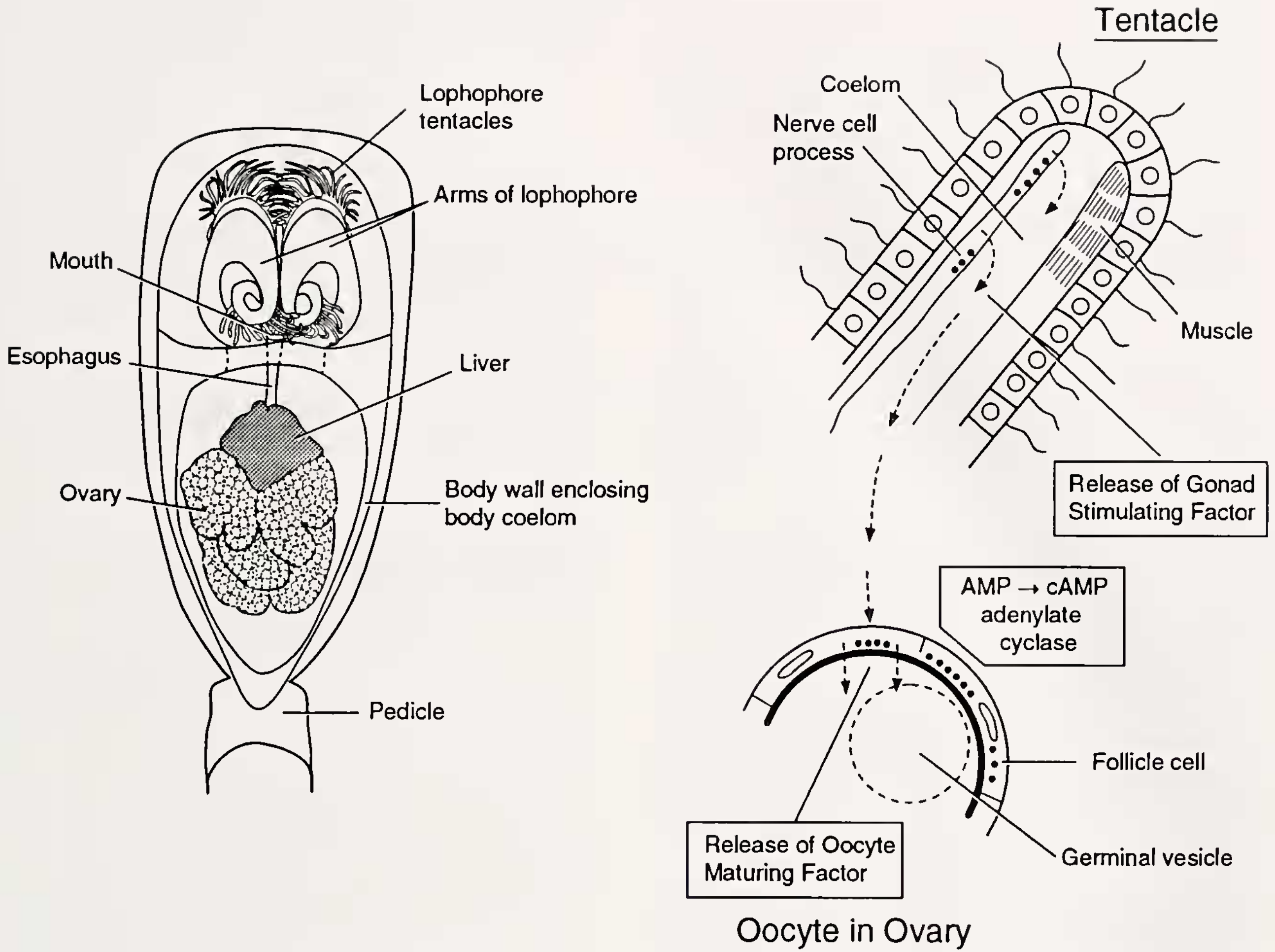

Figure 5. (Left) Diagram of the anterior region of *Glottidia* with one of its valves removed, showing the relative positions of the lophophore and ovary. They are in communication via the animal's coelomic space. (Upper right) Enlarged diagram of lophophore tentacle showing the release of gonad-stimulating factor. In this diagram, the factor is shown being released from a nerve cell process. (Lower right) Enlarged view of oocyte in ovary. The gonad-stimulating factor from the lophophore is shown stimulating cAMP synthesis in follicle cells. These cells then produce an oocyte-maturing factor that acts on oocytes, causing germinal vesicle breakdown.

that normal spawning is triggered when a cell type in the lophophore secretes a hormone with oocyte-maturing activity into the body cavity. Storch and Welsch (1976) describe the ultrastructure of the lophophore and tentacles of the related genus *Lingula*, and Hay-Schmidt (1992) describes the larval lophophore, with special reference to the nervous system, in *Lingula* and *Glottidia*. At present, it is not clear what cell types in the lophophore produce the oocyte-maturing activity. The lophophore is well innervated, and a neurosecretory product is certainly a possibility. In polychaetes and asteroids nerve cells produce hormones that play a role in oocyte maturation. The lophophore hormone then acts on a somatic cell in the ovary, presumably the follicle cell, perhaps increasing its cAMP levels. This leads to the release of another hormone that acts over a much smaller distance on large oocytes to trigger their maturation. Figure 5 outlines this hypothetical endocrine pathway. The oocyte-maturing hormone from the lophophore may also act on the follicle cells, causing them to retract, thereby facilitating oocyte shedding from the ovary. The presence of oocytes in the coelomic cavity may provide an impetus for spawning. Even though no experiments are reported here on spawning in males, the oocyte-maturing hormone is also found in the male lophophore, raising the possibility that this hormone also functions to cause sperm shedding into the coelom and spermiation.

At present, there is no operational way of distinguishing between the oocyte-maturing component of lophophore extract and the biologically active cAMP derivatives.

Cyclic AMP is not degraded by boiling (Sutherland, 1972) or by protease digestion, and its molecular weight is below 2000. Even though the possibility exists that the oocyte-maturing substance produced by the lophophore is identical to cAMP, our knowledge of the context in which cAMP functions in animal cells indicates that this is probably not the case. The oocyte-maturing activity of the lophophore clearly acts intercellularly, whereas cAMP functions intracellularly. The cellular slime mold *Dictyostelium* is the only example that I know of where cAMP functions in an extracellular context as a hormone (Gerisch, 1989). In this case, the cells that receive the signal have cAMP receptors on their surface membranes. Cyclic AMP cannot be the hormone in *Glottidia* because of the large quantities (100 m*M*) needed to give even a marginal oocyte-maturation response. If a cAMP derivative that could diffuse through a lipid bilayer were to function as a hormone, the cells in the lophophore that make the hormone would have a problem storing it. The only way to establish the identity of the lophophore hormone that acts on the gonad and the ovarian hormone that induces oocyte maturation is to purify these substances and carry out the appropriate chemical and structural studies on the molecules. The hypothetical role of lophophore hormone in raising cAMP levels in somatic cells in the ovary can be tested by measuring cAMP levels as a function of hormone treatment.

This study provides a way of obtaining fertilizable oocytes from inarticulate brachiopods at times other than natural spawning, thereby opening up the way for studies on embryogenesis and the early larvae of these animals.

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